#6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Art Unit:
KNUDSEN et al	
	Washington, D.C.
U.S. App. No.: 09/987,108	
	February 6, 2002
National Filing Date: November 13, 2001	
For: BIOSENSOR	Docket No.: KNUDSEN=1A

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please add the following new claim:

67. (Amended) The construct according to 27, wherein the signal molety comprises (6-bromoacetyl-2-dimethylaminonaphthalene) BADAN.

Please amend claims 32, 46, 55, 57, and 65 as follows:

32. (Amended) The construct according to claim 31, wherein the second signal moiety is selected from the group consisting of acrylodan; 5-dimethylaminonaphthalene-1-sulfonyl aziridine (danzyl aziridine); 4-[N-[2-iodacetoxy)ethyl]-N-methylamino]-7-nitrobenz-2-oxa 1,3 diazole ester (IANBDE); 4-[N-[2-

iodoacetoxy)ethyl]-N]methylamino]-7-nitrobenz-2-oxa 1,3 diazole amide (IANBDA); 6-acryloyl-2-dimethylaminonaphthalene (acrylodan); N-(7-chlorobenz-2-oxa-1,3-diazyl-4-yl)sulfonyl morpholine; 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride); didansyl-L-cystine; N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD amide); 7-fluorobenz-2-oxa-1,3-diazole-4-sulfonamide (ABD-F); 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD fluoride);2-(4'-(iodoacetamido)anilino)naphthalene-6-sulfonic acid, sodium salt (IAANS); 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS); 2-(4'-maleimidylanilino)naphthalene-6-sulfonic acid (MIANS); N-(1-pyreneethyl)iodoacetamide; N-(1-pyrene)iodoacetamide; N-(1-pyrene)maleimide; N-(1-pyrenemethyl)iodoacetamide (PMIA amide); 1-pyrenemethyl iodoacetate (PMIA ester); N-(1-pyrenepropyl)iodoacetamide); 1-(2,3-epoxypropyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium trifluoromethanesulfonate (PyMPO epoxide); erythrosin-5-iodoacetamide; fluorescein-5-maleimide; 5-iodoacetamidofluorescein (5-IAF); 6-iodoacetamidofluorescein (6-IAF); 1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium methanesulfonate (PyMPO maleimide); Oregon Green™ iodoacetamide "mixed isomers"; tetramethylrhodamine-5-iodoacetamide (5-TMRIA) "single isomer"; tetramethylrhodamine-5-maleimide "single isomer"; tetramethylrhodamine-6-maleimide "single isomer"; Texas Red® C_5 bromoacetamide; and Texas Red® C_2 maleimide.

46. (Amended) A kit for detection of the concentration of a hydrophobic Coenzyme A ester in a sample comprising

i) at least a first construct according to claim 27,

ii) a sample compartment for application of the sample.

55. (Amended) A kit for detection of the concentration of a hydrophobic Coenzyme A ester in a sample comprising

i) at least first and second constructs according to claim 27,

ii) a sample compartment for application of the sample.

57. (Amended) The kit according to claim 55, wherein each construct has a K_D with respect to at least one species or a group of species of hydrophobic coenzyme A esters, which is substantially lower than the K_D of the other construct(s) with respect to this species or group of species.

65. The method according to claim 60, wherein step iii) comprises diluting a sub-sample of the solvent comprising the free fatty acids in a reaction mixture and performing a method of determining the concentration of free unbound hydrophobic Coenzyme A ester in a sample comprising the steps of

i) providing a hydrophobic Coenzyme A binding construct exhibiting a first signal when unbound and exhibiting a measurably different second signal when bound to a hydrophobic Coenzyme A ester,

ii) contacting the sample with the labeled hydrophobic Coenzyme a binding construct,

iii) allowing at least one species of unbound free hydrophobic Coenzyme A ester to bind to the hydrophobic Coenzyme A binding construct forming a complex comprising a hydrophobic Coenzyme A ester and the hydrophobic Coenzyme A binding construct,

iv) detecting a signal from the complex,

v) correlating the signal to the concentration of at least one species of hydrophobic Coenzyme A ester in the sample.

REMARKS

The above amendments to the claims are being made in order to eliminate multiple dependency and for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with Markings to Show Changes Made**."

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 67 has been added.

32. The construct according to claim **[26]** 31 wherein the second signal moiety is selected from the group **[of claim 30]** consisting of acrylodan; 5-dimethylaminonaphthalene-1-sulfonyl aziridine (danzyl aziridine); 4-[N-[2-iodacetoxy)ethyl}-N-methylamino]-7-nitrobenz-2-oxa 1,3 diazole ester (IANBDE); 4-[N-[2-iodoacetoxy)ethyl]-N]methylamino]-7-nitrobenz-2-oxa 1,3 diazole amide (IANBDA); 6-acryloyl-2- dimethylaminonaphthalene (acrylodan); N-(7-chlorobenz-2-oxa-1,3-diazyl-4-yl)sulfonyl morpholine; 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride); didansyl-L-cystine; N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD amide); 7-fluorobenz-2-oxa-1,3- diazole 4-sulfonamide (ABD-F); 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD fluoride);2-(4'-(iodoacetamido)anilino)naphthalene-6-sulfonic acid, sodium salt (IAANS); 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS); 2-(4'-maleimidylanilino)naphthalene-6-sulfonic acid (MIANS); N-(1-pyreneethyl)iodoacetamide; N-(1-pyrene)iodoacetamide; N-(1-pyrene)maleimide; N-(1-pyrenemethyl)iodoacetamide (PMIA amide); 1-pyrenemethyl iodoacetate (PMIA ester); N-(1-pyrenepropyl)iodoacetamide); 1-(2,3-epoxypropyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium trifluoromethanesulfonate (PyMPO epoxide); erythrosin-5-iodoacetamide; fluorescein-5-maleimide; 5-iodoacetamidofluorescein (5-IAF); 6-iodoacetamidofluorescein (6-IAF); 1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium methanesulfonate (PyMPO maleimide); Oregon Green™ 488 iodoacetamide "mixed isomers"; tetramethylrhodamine-5-iodoacetamide (5-TMRIA) "single isomer"; tetramethylrhodamine-5-maleimide "single

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 67 has been added.

32. The construct according to claim **[26]** 31 wherein the second signal moiety is selected from the group **[of claim 30]** consisting of acrylodan; 5-dimethylaminonaphthalene-1-sulfonyl aziridine (danzyl aziridine); 4-[N-[2-iodacetoxy)ethyl}-N-methylamino]-7-nitrobenz-2-oxa 1,3 diazole ester (IANBDE); 4-[N-[2-iodoacetoxy)ethyl]-N]methylamino]-7-nitrobenz-2-oxa 1,3 diazole amide (IANBDA); 6-acryloyl-2- dimethylaminonaphthalene (acrylodan); N-(7-chlorobenz-2-oxa-1,3-diazyl-4-yl)sulfonyl morpholine; 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride); didansyl-L-cystine; N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD amide); 7-fluorobenz-2-oxa-1,3- diazole 4-sulfonamide (ABD-F); 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD fluoride);2-(4′-(iodoacetamido)anilino)naphthalene-6-sulfonic acid, sodium salt (IAANS); 5-(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (1,5-IAEDANS); 2-(4′-maleimidylanilino)naphthalene-6-sulfonic acid (MIANS); N-(1-pyreneethyl)iodoacetamide; N-(1-pyrene)iodoacetamide; N-(1-pyrene)maleimide; N-(1-pyrenemethyl)iodoacetamide (PMIA amide); 1-pyrenemethyl iodoacetate (PMIA ester); N-(1-pyrenepropyl)iodoacetamide); 1-(2,3-epoxypropyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium trifluoromethanesulfonate (PyMPO epoxide); erythrosin-5-iodoacetamide; fluorescein-5-maleimide; 5-iodoacetamidofluorescein (5-IAF); 6-iodoacetamidofluorescein (6-IAF); 1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium methanesulfonate (PyMPO maleimide); Oregon Green™ 488 iodoacetamide "mixed isomers"; tetramethylrhodamine-5-iodoacetamide (5-TMRIA) "single isomer"; tetramethylrhodamine-5-maleimide "single

isomer"; tetramethylrhodamine-6-maleimide "single isomer"; Texas Red® C_5 bromoacetamide; and Texas Red® C_2 maleimide.

46. A kit for detection of the concentration of a hydrophobic Coenzyme A ester in a sample comprising

i) at least a first construct according to [claims 26-46] claim 27,

ii) a sample compartment for application of the sample.

55. [The kit according to claim 46] A kit for detection of the concentration of a hydrophobic Coenzyme A ester in a sample comprising

i) at least first and second constructs [comprising a second hydrophobic-Coenzyme A ester binding construct] according to [claims 26-45] claim 27,

ii) a sample compartment for application of the sample.

57. The kit according to claim 55 **[or 56]**, wherein each construct has a K_D with respect to at least one species or a group of species of hydrophobic coenzyme A esters, which is substantially lower than the K_D of the other construct(s) with respect to this species or group of species.

65. The method according to claim 60, wherein step iii) comprises diluting a sub-sample of the solvent comprising the free fatty acids in a reaction mixture and performing a method [according to claim 1] of determining the concentration of free unbound hydrophobic coenzyme A ester in a sample comprising the steps of

i) providing a hydrophobic Coenzyme A binding construct exhibiting a first signal when unbound and exhibiting a measurably different second signal when bound to a hydrophobic Coenzyme A ester,

ii) contacting the sample with the labeled hydrophobic Coenzyme a binding construct,

iii) allowing at least one species of unbound free hydrophobic Coenzyme A ester to bind to the hydrophobic Coenzyme A binding construct forming a complex comprising a hydrophobic Coenzyme A ester and the hydrophobic Coenzyme a binding construct,

iv) detecting a signal from the complex,

v) correlating the signal to the concentration of at least one species of hydrophobic Coenzyme A ester in the sample.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Conf. No.: 6445
KNUDSEN et al	)	Examiner:
Appln. No.: 09/987,108	)	Washington, D.C.
Filed: November 13, 2001	)	February 6, 2002
For: BIOSENSOR	)	Atty.Docket: KNUDSEN=1A

RESPONSE TO "SEQUENCE LISTING" REQUIREMENT

Honorable Commissioner of Patents
Washington, D.C. 20231

Sir:

In response to the Notice to Comply included in the Notice to File Missing Parts of Nonprovisional Application, mailed December 6, 2001, please amend the application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at line 3 of page 10 with the following rewritten paragraph:

--Fig 1. Alignment of 30 ACBP sequences (numbered consecutively from top to bottom as SEQ ID NOs:1-30). The alignments are with respect to the bovine sequence (SEQ ID NO:1) with residues Ser 1 to Ile 86. The lengths of the other sequences are indicated as a subscript after the last residue shown and the four helices of bovine ACBP are shown as boxes above the sequences. Conserved Class 1 residues are present

in 18 out of the 21 l- and b-ACBPs and are highlighted by black boxes. Conserved class 2 residues are hydrophobic residues (either M/L/H/P/A/F/Y/V/I) in all l- and b-ACBP sequences and in at least 27 out of all 30 sequences and are highlighted by grey boxes. Cysteines are in white text in grey boxes. Yeast(1) is from Saccharomyces cervisiae and Yeast(2) from Saccharomyces monoasensis and from Saccharomyces pastoranis (identical).--

Please replace Table 1 beginning at line 18 of page 48 with the following rewritten Table 1:

--Table 1 Primers used for site directed mutagenesis of bovine ACBP

Mutation	Sequence
M24C	
Upsteam	5'-TGCTTGTTCATCTACTCTCACTACAAG (SEQ ID NO:31)
Downstream	5'-TTCTTCGTCGGCCGGCTTGGTCTTC (SEQ ID NO:32)
M46C	
Upsteam	5'-TGCTTGGACTTCAAGGGTAAGGCTAAG (SEQ ID NO:33)
Downstream	5'-CCCGGGTCTTTCGGTGTTGATGTC (SEQ ID NO:34)
A53C	
Upsteam	5'-TGCAAGTGGGACGCTTGGAACGAATTG (SEQ ID NO:35)
Downstream	5'-CTTACCCTTGAAGTCCAACATCCC (SEQ ID NO:36)

--

Please replace the paragraph beginning at line 13 of page 57 with the following rewritten paragraph:

--Recombinant *E. coli* fatty acyl-CoA synthetase was expressed as a N-terminal GST-fusion protein. The open reading frame of the *E. coli* fatty acyl-CoA synthetase was amplified using the pN3576 plasmid as template (Black et al., 1997) and specific oligonucleotides 5'-CACGGATCCATGAAGAAGGTTTGGCTTAACC-3' (SEQ ID NO:37) and 5'-CACGAATTCTCAGGCTTTATTGTCCACTTTG-3' (SEQ ID NO:38), carrying either a *Bam*H1 and *Eco*R1 restriction site (underlined),

respectively. The Expand High Fidelity PCR System was used as described by the manufacturer (Roche). The PCR product was digested with *Eco*R1 and *Bam*H1 and ligated into the pGEX-2TK vector (Pharmacia) using standard techniques. The recombinant GST-fusion protein was expressed in *E. coli* BL21(DE3) strain and purified essentially as described by the manufacturer (Pharmacia), except that CoA (10 mM) was included in all buffers including the elution buffer.--

IN THE SEQUENCE LISTING

Please substitute the attached Sequence Listing, numbered as pages for the Sequence Listing previously submitted.

REMARKS

1. Applicants hereby submit the following:

[ ] a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification as directed above;

[XX] an amendment to the paper copy of the "Sequence Listing" submitted on November 13, 2001, the amendment being in the form of substitute sheets;

[XX] the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein;

[ ] pursuant to §1.821(e), reference is made to the computer readable form filed on , in USSN , which presents the identical Sequence information, the use of which is now requested, in lieu of submitting a new computer readable form; and/or

[ ] a substitute computer readable form to replace one found to be damaged or unreadable.

[XX] 2. The description has been amended to comply with §1.821(d).

3. The undersigned attorney or agent hereby states as follows:

(a) this submission is not believed to include new matter [§1.821(g)];

(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [§1.821(f) and §1.825(b)];

(c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [§1.825(a)]; and

(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [§1.825(d)].

4. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of

"Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free

sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

BROWDY AND NEIMARK
Attorneys for Applicant(s)

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Iver P. Cooper
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at line 3 of page 10 has been amended as follows:

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Upsteam	5'-TGCAAGTGGGACGCTTGGAACGAATTG (SEQ ID NO:35)
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either a *Bam*H1 and *Eco*R1 restriction site (underlined), respectively. The Expand High Fidelity PCR System was used as described by the manufacturer (Roche). The PCR product was digested with *Eco*R1 and *Bam*H1 and ligated into the pGEX-2TK vector (Pharmacia) using standard techniques. The recombinant GST-fusion protein was expressed in *E. coli* BL21(DE3) strain and purified essentially as described by the manufacturer (Pharmacia), except that CoA (10 mM) was included in all buffers including the elution buffer.